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James B. Lackey

Public Health Service
Cincinnati, Ohio

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MEMORANDUM

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The microscopic flora and fauna exclusive of bacteria, of natural waters, prepared waters from water purification plants, and raw and treated sewage, offer a variety of information relative to those waters. The organisms frequently indicate the hardness or softness of the waters involved; the nature of the acidity, if acid; the region from which the water is derived; the degree of fertilization of the water; the source of tastes and odors; the degree of effectiveness of purification and filtration; the condition of a sewage treatment plant; and sometimes what specific trade wastes are present. In a lake they offer some information as to what depth is represented by a sample and whether or not the lake is stratified. Pollution can be classified as recent or remote, gross or light. Biologic conditions may be shown to be balanced or unbalanced. In short, it is possible to take a sample of water and from a careful qualitative and quantitative analysis of its organisms, accurately deduce many facts about the source of the sample.

Such deductions to be accurate presuppose two things. The first of these is that the groups of organisms studied be known and considered in their entirety and that no large numbers be omitted because unfamiliar to the worker. Thus the student should have a considerable taxonomic and ecologic knowledge of the groups encountered, or of the groups with which it is proposed to work. The second is that the sample in question be

truly representative of enough volume to contain large numbers of the organisms which it is desired to study, properly taken, and properly prepared.

Concerning the first postulate there is much to be said for working with microscopic organisms. This is frequently referred to as plankton study, but actually the plankton worker considers many non-plankton types as well, for those benthic forms that are not firmly attached to the bottom or do not burrow into the mud, are frequently encountered in stream samples, having been swept into suspension by the current. In lakes it is possible to more clearly separate plankton and benthos.

Only two plant groups are microscopic. The free living fungi are rare in lakes, streams and reservoirs but are abundant in sewage treatment plants. Parasitic fungi, such as those often encountered on diatoms or Pandorina, are difficult to work with, and only a few can be referred to as common. Algae are abundant and if we consider the flagellated chlorophyll containing forms, are present in variety except in the very coldest months. Even then there may be huge blooms of forms such as Chlamydomonas under the ice in lakes. Their great diversity sometimes calls for specialized taxonomic knowledge, as of the diatoms or the desmids.

Several groups of animals are predominantly microscopic. Protozoa head the list. Characteristically bottom dwellers, there are nevertheless distinct plankton species in each of the three free living groups. This is exceptionally well shown in surface samples from the high seas.

Nematodax worms are probably next in variety, but are found mostly in bottom samples, and in sewage treatment plants. Rotifers, at least in small samples, probably outnumber the nematodes, and many species are truly planktonic. They too, are common to sewage treatment plants.

Other microscopic animals are less abundant in random samples, unless the sample is large. There are a few turbellarian worms which are common, especially in bottom samples, and likewise certain oligochalte worms. Of these last, Dero, Aeslosoma, Tubifex, Limnodrilus and one or two others are of considerable value as indicator organisms, and are easily cultivated under laboratory conditions. Gastrotrichs are sometimes abundant but almost always belong to the genus Chactonotus. Water bears, (Tardigrada) occur infrequently in bottom samples and in sewage treatment plants. They are arthropods, but the most abundant arthropods are the water fleas and water mites - Copepoda, Ostracoda, etc. These are seldom abundant in small samples, and except for the red water mites are usually planktonic.

Advantages of working with microscopic forms might be summed up as follows:

1. Only small samples need be taken. Usually 250 to 500 mls. are sufficient. As an example, a liter of surface water from far out in the Atlantic contained 70 identifiable species, totalling 8117 individuals.
2. Many samples can be handled and transported on a single trip.

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3. No large sampling crew, and no cumbersome gear is necessary unless micro-crustacea are wanted, even a plankton net is unnecessary.

4. If iced, samples can be brought into the laboratory with organisms alive and many are easily cultivated. For example, Chlamydotheca unispinosa, an ostracod some 3 mm. in length, was accidentally brought into the laboratory several years ago, but grew well and has been a useful laboratory animal.

5. A large and representative population is sampled.

6. The catch may be measured in drops, i.e. 5 drops = 100 mls. of raw water, and a single drop may be counted under a 25 mm. cover glass, thus eliminating time consuming filtering, and a counting chamber.

Regarding the second postulate, i.e. sampling, the following points have been common practice:

1. Stream samples are taken in the current, a few inches below the surface. The best samples are secured with a wide mouth sampler. Lake or pond samples are taken either just above the lake outlet or on the side toward which the wind is blowing unless a series of vertical samples is desired, and at the surface if a single sample is taken. Of course a sample may be desired from a particular spot.

2. If the sample is to be examined unkilled, it is put into an ice box of some sort, during transport and until use. The floor of an automobile frequently gets hot enough to greatly injure a sample.

3. For general preservation, 3 to 5 mls., no more, of formalin, either c.p. or commercial grade, per 100 mls. of sample is added.

Almost all organisms are recognizably preserved for as long as 5 years by this method. Exceptions are naked rhizopods, naked flagellates, some ciliates and a few worms. Fresh samples are always best. Bog samples containing much humic (tannic?) acid are sometimes discolored after formalin preservation.

4. Storage is always in the dark. A cool place is best.

5. A slip of hard paper (not glazed) containing data <u>in pencil</u> is put into the sample. Any data is useful, but location, amount of sample and date are necessary. Temperature, pH, any other data may be included.

6. Concentration is by centrifuging for living material. Conical and 50 ml. tubes are used, at a rate of 2000 to 3000 r.p.m. for 5 minutes. This distorts almost no organisms, whereas high speeds wreck such organisms as cryptomonas, amoebas, many flagellates and ciliates. For decantation from such tubes the best practice is to take a 50 ml. volumetric pipette, cut off the delivery point until the opening is about 1 mm. across, then attach a 50 ml. bulb to the large end. Supernatant water down to the last 10 or 15 drops (containing the catch) may be sucked off with this, with no disturbance of the catch. But with some samples (pelagic ocean samples for example) the catch may be about one or two drops per liter of preserved material. For the final withdrawal of water, a pipette with a medicine dropper bulb is used.

Concentration of preserved material may be preceded by storage - ten days to months. In these cases it is easy to pour off all but the final 50 mls. if the storage is in jars with wide mouths. Fruit jars are suitable. As many as 50 of these may be set away in a dark, cool place and, if the material is to be shipped away, only small containers are necessary to hold the catch, saving space and shipping charges.

7. Volumetric ratios must be taken into account, but it is a very simple matter to rate the catch in drops. If it amounts to 10 drops for a 400 ml. sample, one drop contains the organisms in 40 mls.

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